Activation of monocytes by three OspA vaccine candidates: lipoprotein OspA is a potent stimulator of monokines

Häupl, T ; Landgraf, S ; Netusil, P ; Biller-Andorno, Nikola ; Capiau, C ; Desmons, P ; Hauser, P ; Burmester, G-R

DOI: https://doi.org/10.1111/j.1574-695x.1997.tb01068.x

ELSEVIER

FEMS Immunology and Medical Microbiology 19 (1997) 15–23

FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Activation of monocytes by three OspA vaccine candidates: lipoprotein OspA is a potent stimulator of monokines

T. Häupl [a,*], S. Landgraf [b], P. Netusil [b], N. Biller [b], C. Capiau [c], P. Desmons [c], P. Hauser [c], G.-R. Burmester [a]

[a] *Department of Medicine III, Charité, Humboldt University, Schumannstraße 20/21, 10089 Berlin, Germany*
[b] *Department of Medicine III, University of Erlangen, Erlangen, Germany*
[c] *SmithKline Beecham Biologicals, Rixensart, Belgium*

Received 15 May 1997; accepted 27 May 1997

Abstract

The outer surface protein (Osp) A of *Borrelia burgdorferi* is the first Lyme antigen to be tested in a vaccine for humans. Three forms of OspA vaccine candidates were investigated by the induction of the cytokines interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, IL-10 and interferon (IFN)-γ as markers of monocyte activation and immune stimulation: lipidated OspA (L-OspA), non-lipidated OspA (NL-OspA), and a fusion protein of 81 amino acids of the nonstructural protein 1 of influenza virus with OspA (NS1-OspA). All OspA preparations induced IL-1β, IL-6 and TNF-α in a concentration-dependent manner with peak levels at 12–24 h. These cytokines were entirely derived from the monocyte fraction. In peripheral blood mononuclear cells from 10 healthy donors, L-OspA at 10 μg ml^{-1} induced up to 4-fold more IL-1β, IL-6, and TNF-α than the other OspA preparations ($P \leq 0.0068$), followed by NS1-OspA, which was still superior to NL-OspA. L-OspA also induced high levels of IL-10 within 24 h but no significant amounts of IFN-γ. This superior stimulating activity of L-OspA on unstimulated monocytes predominantly depended on N-terminal lipidation of OspA. Similarities to other lipoproteins and synthetic lipopeptides suggest that lipidation confers adjuvant properties on OspA. High induction of IL-10 by L-OspA further suggested a negative feedback on monocyte activation by the lipidated form. The in vitro results are in line with in vivo results in mice, monkeys and humans and indicates that lipoprotein OspA has the best potential for induction of a protective effect in humans, compared to non-lipidated antigens.

Keywords: Lyme vaccine; OspA; Cytokine

1. Introduction

Lyme disease, caused by infection with *Borrelia burgdorferi*, can become chronic due to long term persistence of spirochetes especially in immunologically privileged sites and bradytrophic tissues, where antibiotics may fail to eradicate the pathogen [1,2], and/or by infection induced immunopathology.

Once *Borrelia* organisms have entered the human body after a tick bite, several evasion mechanisms of the spirochete support persistent infection. Besides invasion of immunoprivileged sites like dense connective tissue [2,3], there is an unusual phagocytic

* Corresponding author. Tel.: +49 (30) 2802-8286; fax: +49 (30) 2802-8300; e-mail: thomas.haeupl@rz.hu-berlin.de

PII S0928-8244(97)00046-1

uptake [4] with ultrastructural alterations in phagocytes [5], which may be associated with insufficient antigen presentation and specific immune activation [6]. Furthermore, modulation in surface antigen expression may support immunological escape. The outer surface protein (Osp) A is of particular interest. OspA was shown to be expressed on spirochetes in the midgut of non-feeding ticks, but disappeared during blood feeding and initial infection [7]. Vaccination with OspA induced protective immunity in mice challenged by needle infection or by feeding of infected ticks [8–11]. Pre-existing anti-OspA antibodies in mice eliminated the OspA expressing spirochetes inside the feeding tick [7,12,13]. Therefore vaccination with OspA antigen is a promising strategy for protection against transduction of *B. burgdorferi* by tick bites and subsequent disease. There is concern, however, about the safety of a Lyme disease vaccine with regard to the possibility that certain *B. burgdorferi* antigens or vaccine formulations may trigger a potentially harmful immune response, if infection occurs despite previous vaccination or if infection is preexisting [14]. Furthermore, this immunopathology may be associated with certain major histocompatibility antigens [15] and/or OspA specific T cell responses in treatment resistant Lyme arthritis [16–18].

For successful induction of a protective immune response, immunogenicity and adjuvanticity are important characteristics for a vaccine. Molecular structure and composition of the vaccine may influence the type of immune reaction towards a predominantly humoral or cellular response. Immunogenicity of OspA protein in humans has been described earlier and mapping of the epitopes recognized by naturally induced antibodies [19,20] and OspA specific T cells [17,20,21] was performed. Immunogenicity can be further increased by the use of fusion proteins between highly immunogenic peptides and the antigen of interest [9,22–25]. Adjuvanticity can be achieved by the addition of a separate adjuvant (e.g., aluminum hydroxide) or by modification of the vaccine candidate antigen (lipidation) [26–29]. Activation of monocytes is an important measure of the potential of adjuvants. In animal experiments induction of anti-OspA antibodies and specific T cell proliferation have been analyzed after vaccination with lipidated, non-lipidated or fusion proteins. Lipidated OspA was shown to be the most potent inducer, whereas non-lipidated OspA was unable to trigger specific responses in unprimed animals [26]. In mice and human monocyte/macrophage preparations, release of interleukin (IL)-1β, IL-6 or tumor necrosis factor (TNF)-α by *B. burgdorferi* spirochetes or purified or synthetic spirochetal lipoprotein was analyzed, indicating potent induction by lipoproteins [30–33]. However, a comparative study of the different forms of OspA vaccine candidates, including lipidated, non-lipidated and fusion proteins, has not been done yet.

2. Materials and methods

2.1. Donors and cell preparation

Peripheral blood mononuclear cells (PBMC) and monocytes were derived from 12 healthy donors. PBMC were separated from 10 healthy donors by Ficoll-Hypaque density centrifugation, washed twice with PBS and resuspended in RPMI 1640 supplemented with 15 mM HEPES, 4 mM L-glutamine, 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10% (v/v) autologous serum. Monocytes were separated from lymphapheresis material of two healthy donors by two steps of density centrifugation (Ficoll-Hypaque and 51% Percoll) and subsequent depletion of T cells by E-rosetting with neuraminidase treated sheep erythrocytes and centrifugation over Ficoll-Hypaque. Depleted T cells were recovered by treatment with double distilled water for 30 s and 3–5 steps of washing in PBS.

2.2. Antigens and B. burgdorferi spirochetes

The recombinant OpsA forms, shown in Fig. 1, were expressed in *Escherichia coli*. Lipidated OspA (L-OspA) was synthesized in *E. coli* by expression of the complete OspA gene sequence including the codons for the signal peptide of lipidation. To produce non-lipidated OspA (NL-OspA), the codons for the signal peptide had been removed. In the third preparation OspA protein was fused to a 81 amino acid segment of non-structural protein 1 of influenza (NS1-OspA) [24]. These OspA forms were produced and purified in the laboratories of SmithKline

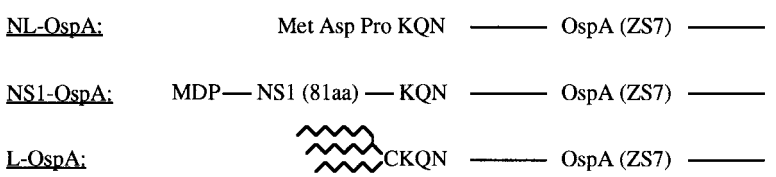

Fig. 1. Formulations of the three OspA preparations used with important amino acids given in one letter code.

Beecham Biologicals. Lipopolysaccharide (LPS) contamination was determined by the Limulus assay and was comparable for all preparations: less than 10 pg μg^{-1} protein. Tetanus toxoid as a reference antigen was kindly provided by Behring Werke, Marburg. *B. burgdorferi* spirochete strain ZS7, the strain from which the OspA sequence was derived, was cultured in BSK medium [2]. Bacteria were harvested when motility was still optimal by centrifugation at $1800\times g$ and subsequently one washing step with RPMI 1640. Aliquots (100 µl) of 4×10^9 cells ml^{-1} were frozen at −70°C until stimulation assays were performed.

2.3. Stimulation and cytokine assays

Stimulation of cells with antigens was performed in 24 well plates and 1.0 ml of RPMI 1640 culture medium supplemented with 15 mM HEPES, 4 mM L-glutamine, 10% v/v autologous serum. Monocytes or PBMC (1×10^6 ml^{-1}) were incubated with 1×10^6 cells ml^{-1} of *B. burgdorferi* strain ZS7 spirochetes, various concentrations from 1 ng ml^{-1} to 10 µg ml^{-1} of L-OspA, NL-OpsA, NS1-OpsA or tetanus toxoid (TT), and 1 pg ml^{-1} to 10 ng ml^{-1} LPS (kindly provided by Dr. Galanos, Max-Planck Institut, Freiburg, Germany). Stimulation assays were incubated at 37°C for up to 96 h. Subsequently, supernates were harvested by transfer of each culture to a microfuge tube, centrifugation at $1000\times g$ for 5 min and transfer to new microfuge tubes in 125 µl aliquots. Samples were frozen at −20°C until cytokine determination was performed. For each cytokine assay, a new aliquot was thawed and remainders were discarded.

Culture supernates and appropriate dilutions were tested in commercial ELISA systems for IL-1β, IL-6, TNF-α (Quantikine, R and D Systems, Minneapolis, MN, USA), IL-10 (Dianova, Hamburg) and interferon (IFN)-γ (Holland Biotechnology, The Netherlands) according to the manufacturers' protocols.

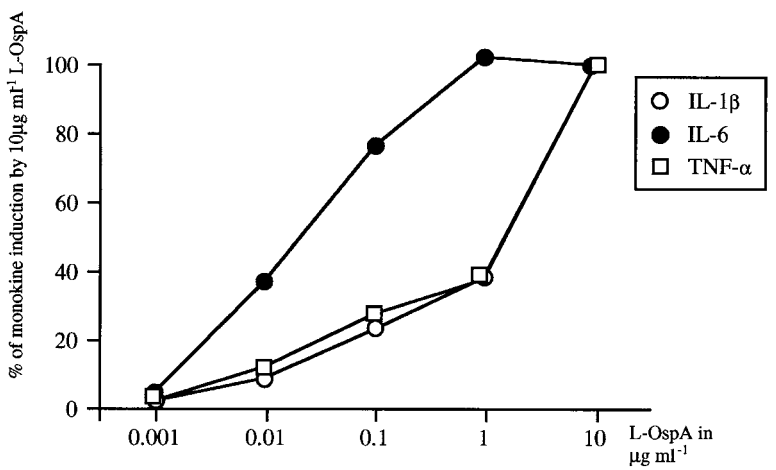

Fig. 2. Concentration-dependent induction of monokines by L-OspA in PBMC of a normal donor, representative of experiments with three other normal donors and comparable results. Results are given in % of monokine induction by 10 µg ml^{-1} of L-OspA, equivalent to 211 pg ml^{-1} of IL-1β, 673 pg ml^{-1} of TNF-α and 8466 pg ml^{-1} of IL-6.

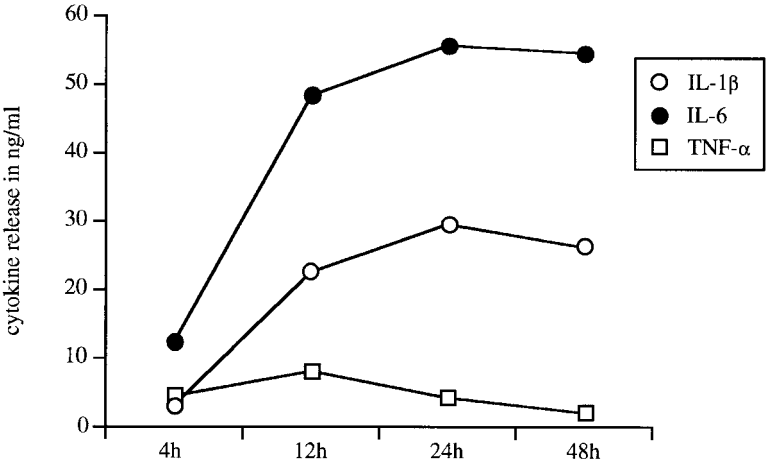

Fig. 3. Kinetics of monokine release after stimulation of peripheral blood monocytes of a normal donor with 10 μg ml^{-1} of L-OspA.

3. Results

3.1. Concentration-dependent stimulation of cytokines

Two representative PBMC preparations were stimulated with increasing amounts of L-OspA, NL-OspA and NS1-OspA from 1 ng ml^{-1} to 10 μg ml^{-1} of protein. Supernates were harvested after 24 h and cytokines were measured in ELISA systems specific for IL-1β, IL-6 and TNF-α. As shown in Fig. 2 representing one normal donor (ND) after stimulation with L-OspA, there was a dose dependent induction of all three monokines. Highest levels were released after L-OspA stimulation, followed by NS1-OspA. Detectable levels of all three cytokines were found after stimulation with as little as 10 ng ml^{-1} of L-OspA, whereas NL-OspA and NS1-OspA were about 100-fold less active, inducing significant levels of cytokine only in concentrations of 1 μg ml^{-1} and higher (data not shown). LPS was measured by the Limulus assay and was comparable for all three preparations and below 10 pg μg^{-1} of OspA protein. In dilution experiments with highly purified LPS, these concentrations did not interfere with our stimulation assay (data not shown).

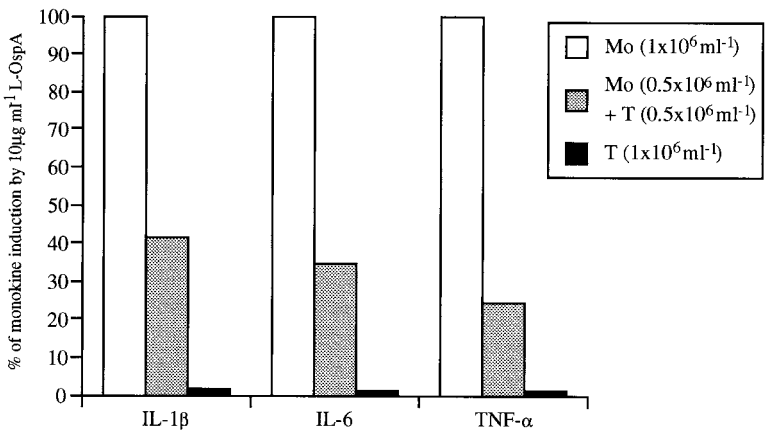

Fig. 4. Induction of monokines by L-OspA depends on concentration of monocytes and is independent of interaction with lymphocytes. Results are given in % of monokine induction by 10 μg ml^{-1} L-OspA in purified monocyte preparations, equivalent to 22 ng ml^{-1} of IL-1β, 48 ng ml^{-1} of IL-6 and 8 ng ml^{-1} of TNF-α.

3.2. Kinetics of cytokine release

Induction of monokines was measured from 4 to 48 h of stimulation with 10 μg ml^{-1} of OspA protein preparations. For this experiment, monocytes were purified from a normal donor. Maximum levels of IL-1β and IL-6 were found after 24 h, whereas TNF-α peaked at 12 h of stimulation. Fig. 3 shows the results after stimulation with L-OspA.

3.3. IL-1β, IL-6 and TNF-α are released by monocytes

To determine the cellular source of the cytokines IL-1β, IL-6 and TNF-α, subpopulations of PBMC were analyzed. From lymphapheresis material of a normal donor, monocytes and non-monocytic cells were separated [4] and stimulated with L-OspA (10 μg ml^{-1}) individually and in equal mixtures for 12 h; results are presented in Fig. 4. None of the three cytokines was detectable in supernates from stimulated T cells. Comparing monokine levels between monocyte preparations and equal mixtures of monocytes and T cells of the same donor, monokine release turned out to be 25–42% of maximum release in monocyte preparations. This indicates that release of IL-1β, IL-6 and TNF-α after L-OspA stimulation depended on the absolute concentration of monocytes. Monokine release was independent of interactions with lymphocytes, which did not significantly alter monokine release.

3.4. Comparison of monokine induction after stimulation with three different OspA forms

PBMC from 10 normal donors were incubated with 10 μg ml^{-1} of L-OspA, NL-OspA and NS1-OspA for 24 h. Additional stimulation experiments with *B. burgdorferi* spirochetes (10^6 ml^{-1}) and TT (10 μg ml^{-1}) served as control. Mean values and standard errors of the mean are illustrated in Fig. 5. L-OspA proved to be the most effective stimulator of all three monokines. This result was highly significant ($P < 0.0001$ for IL-1β, $P \leq 0.0068$ for IL-6, $P \leq 0.0034$ for TNF-α). Interestingly, NS1-OspA induced about 50% of the monokine levels of L-OspA and induced significantly more IL-1β and IL-6 than NL-OspA. Comparing IL-1β and IL-6 release by L-OspA, there was a low but significant correlation ($r = 0.73$; $P = 0.017$). In contrast, TNF-α release did not correlate with any of the other two cytokines ($r \leq 0.25$; $P \geq 0.49$). TT as a non-lipidated control antigen induced about half the IL-1β and IL-6 compared to NL-OspA, whereas TNF-α release was about 20% higher (data not shown). Production of monokines as a response to whole spirochetes was also significant and levels ranged between those of NS1-OspA and L-OspA (Fig. 5).

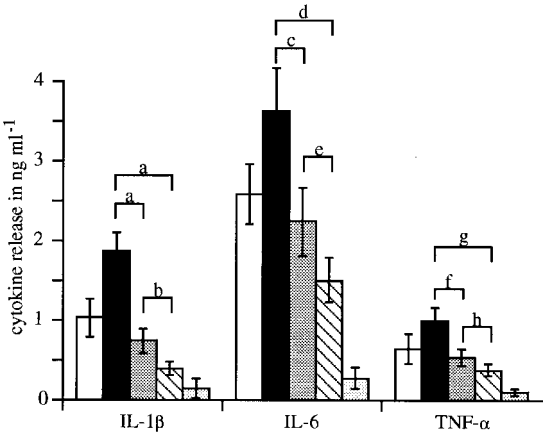

Fig. 5. Induction of monokines by vaccine candidates (black bars L-OspA, cross-hatched bars NS1-OspA, hatched bars NL-OspA) and *B. burgdorferi* spirochetes (white bars ZS7) in PBMC of 10 normal donors. Control values (lightly cross-hatched bars) are derived from incubation of cells without any stimulation. Significances of differences are indicated by letters, corresponding to $P < 0.0001$ (a), $P = 0.026$ (b), $P = 0.0068$ (c), $P = 0.0009$ (d), $P = 0.0019$ (e), $P = 0.0034$ (f), $P = 0.0022$ (g) and $P = 0.15$ (h).

3.5. Release of IL-10 and IFN-γ from PBMC after L-OspA stimulation

PBMC of a normal donor were incubated with L-OspA (10 μg ml^{-1}), ZS7 *B. burgdorferi* spirochetes (1×10^6 ml^{-1}), TT (10 μg ml^{-1}) and LPS (100 ng ml^{-1}). There was considerable induction of IL-10 by L-OspA with a peak at 24 h and a sharp decrease at 48 h (Fig. 6). Other stimulators resulted in much less IL-10 release. Spirochetes and TT also induced peak levels at about 24 h. However, supernates of PBMC exposed to borreliae showed a second increase after 72 and 96 h, whereas after incubation with TT they dropped to baseline levels of IL-10 after 72 h and more. IFN-γ was found in significant

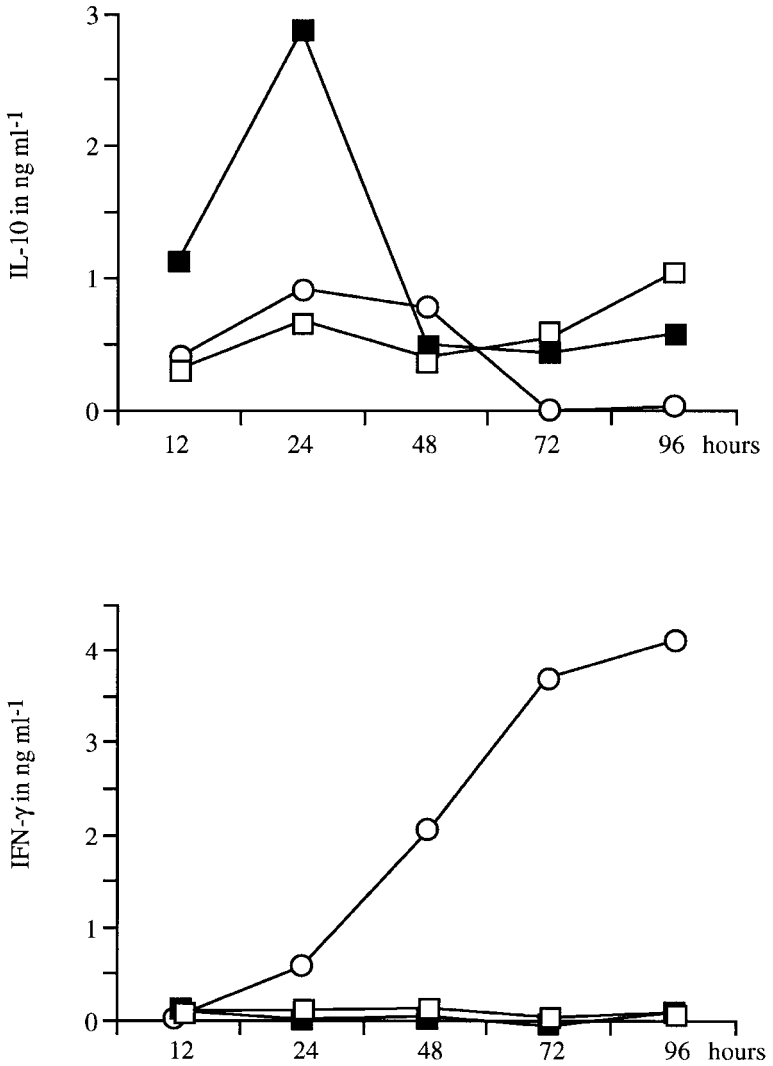

Fig. 6. Kinetics of IL-10 and IFN-γ release by PBMC of a normal donor after stimulation with L-OspA (■), tetanus toxoid (○) and whole spirochetes (□).

levels only after TT stimulation increasing from 24 to 96 h. L-OspA, spirochetes and LPS did not induce any significant levels of this cytokine within 96 h (Fig. 6).

4. Discussion

This study was performed to compare three different forms of OspA vaccine candidates with individual molecular characteristics, two of which (L-OspA and NS1-OspA) had been tested in clinical trials.

Lipidated OspA is a potent inducer of IL-1β, TNF-α and IL-6. Furthermore, this study revealed that L-OspA induced release of these cytokines in a dose dependent fashion with peak levels within 12–24 h and was effective in very low OspA concentrations, at least 100-fold lower than non-lipidated OspA and the NS1 fusion protein.

These cytokines were released exclusively by monocytes and the presence of non-monocytic cells

during monokine stimulation by L-OspA had no significant effects.

L-OspA induced significantly higher levels of IL-10 compared to non-lipidated forms, indicating a self-control of the high stimulatory and inflammatory activity.

At antigen concentrations comparable to a vaccination dose for human individuals, NS1-OspA induced only 50% of the amount of monokines compared to L-OspA but was still superior in IL-1β and IL-6 stimulation compared to NL-OspA.

Lipidation of OspA is responsible for the highly increased potential of monokine induction. This can be concluded from the fact that the two preparations L-OspA and NL-OspA had the same protein sequence except for the very first three amino acids (Fig. 1).

The effect of lipidated proteins on the stimulation of immune cells has been analyzed in several models. Bacterial lipoproteins from *E. coli* or *S. typhimurium* for example are potent B cell mitogens and activate monocytic cells [34–37]. This effect has been ascribed to the lipid component by reduction of the molecular structure to synthetic lipopeptides which stimulated macrophages in tumor cytotoxicity as well as IL-1 production [37]. Furthermore, synthetic lipopeptides were mitogenic for B cells [38] and covalent linkage to antigen elicited antigen specific antibodies [28]. In other studies, influenza virus specific cytotoxic T lymphocytes were primed only with lipidated but not non-lipidated virus derived peptide [39]. As a possible mechanism for these special properties, lipopeptides were shown to integrate into lipid membranes of cells [40,41], where they may exhibit receptor-like functions. All these qualities of lipidated proteins obviously allow a successful vaccination of naive individuals without additional adjuvant [42]. Comparable effects were demonstrated for OspA of *B. burgdorferi*, which is a potent vaccine [26], B cell mitogen [32], and inducer of macrophage activation [33] when lipidated. Given this background, the high potential of L-OspA to induce monokines can be considered an intrinsic adjuvant character dependent on lipidation. This is further supported by at least 100-fold higher activity in monokine induction of L-OspA compared to NL-OspA.

The mode of action of adjuvants has been summarized by Chedid [43], including formation of a depot, presentation of the antigen to immunocompetent cells and production of different lymphokines. The role of lymphokines, especially IL-1, is supported by the observation that a nonapeptide of the IL-1β molecule (163–171) has immunostimulating activities of whole IL-1β devoid of all pro-inflammatory activities and has successfully been used as adjuvant in experimental vaccination studies [44]. High induction of IL-1β and other monokines by the lipoprotein, however, will also result in multiple pro-inflammatory effects, which may explain the increased local side effects in the initial vaccination trials with L-OspA preparations compared to NS1-OspA and NL-OspA [45]. On the other hand, increased release of the anti-inflammatory cytokine IL-10 also by L-OspA may down-regulate this initial monocyte activation thereby limiting the undesired side effects in vaccination.

As a potential complication of a Lyme vaccine the induction of a harmful immune response in certain individuals at risk has been discussed. In humans, this has been suggested by observations that certain HLA-DR groups are associated with chronic Lyme arthritis after intensive antibiotic therapy and negative *B. burgdorferi* specific PCR results [15,46]. Furthermore, in these treatment resistant patients, there was a significantly increased T cell reactivity against OspA and the epitope recognized on OspA has been described [16,17]. In animals, vaccination with whole cell preparations of *B. burgdorferi* spirochetes was associated with more severe arthritis if infection with an antigenetically different *B. burgdorferi* substrain occurred [14]. On the other hand, vaccination with an OspA/glutathione *S*-transferase fusion protein had beneficial effects on pre-established disease in sensitive C3H mice [47], indicating that at least this kind of vaccine formulation does not trigger an aggravating immune response in these animals. Furthermore, in a clinical trial in infected patients, a vaccine containing lipo-OspA on $Al(OH)_3$ was safe and did not induce exacerbated reactions, suggesting that OspA is non-arthritogenic in man [48].

Our results indicate that the improved immunogenicity observed with L-OspA compared to non-lipidated OspA both in mice [26] and in humans [45] is due to its intrinsic adjuvant activity allowing a better activation of monocytes/macrophages. This increased stimulation of monokine secretion appar-

ently does not result in exacerbated reactions in infected mice [47] or humans [48].

Acknowledgments

The authors thank Andreas Krause and Thomas Kamradt for fruitful discussion and reviewing of the manuscript.

References

[1] Steere, A.C. (1989) Lyme disease. New Engl. J. Med. 321, 586–596.
[2] Häupl, T., Hahn, G., Rittig, M., Krause, A., Schoerner, C., Schonherr, U., Kalden, J.R. and Burmester, G.R. (1993) Persistence of *Borrelia burgdorferi* in ligamentous tissue from a patient with chronic Lyme borreliosis. Arthritis Rheum. 36, 1621–1626.
[3] Kimsey, R.B. and Spielman, A. (1990) Motility of Lyme disease spirochetes in fluids as viscous as the extracellular matrix. J. Infect. Dis. 162, 1205–1208.
[4] Rittig, M.G., Krause, A., Häupl, T., Schaible, U.E., Modolell, M., Kramer, M.D., Lutjen Drecoll, E., Simon, M.M. and Burmester, G.R. (1992) Coiling phagocytosis is the preferential phagocytic mechanism for *Borrelia burgdorferi*. Infect. Immun. 60, 4205–4212.
[5] Rittig, M.G., Häupl, T., Krause, A., Kressel, M., Groscurth, P. and Burmester, G.R. (1994) *Borrelia burgdorferi*-induced ultrastructural alterations in human phagocytes: a clue to pathogenicity? J. Pathol. 173, 269–282.
[6] Rittig, M.G., Häupl, T. and Burmester, G.R. (1994) Coiling phagocytosis: a way for MHC class I presentation of bacterial antigens? Int. Arch. Allergy Immunol. 103, 4–10.
[7] de Silva, A.M., Telford, S.R., Brunet, L.R., Barthold, S.W. and Fikrig, E. (1996) *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J. Exp. Med. 183, 271–275.
[8] Schaible, U.E., Kramer, M.D., Eichmann, K., Modolell, M., Museteanu, C. and Simon, M.M. (1990) Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice. Proc. Natl. Acad. Sci. USA 87, 3768–3772.
[9] Fikrig, E., Barthold, S.W., Kantor, F.S. and Flavell, R.A. (1990) Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. Science 250, 553–556.
[10] Simon, M.M., Schaible, U.E., Kramer, M.D., Eckerskorn, C., Museteanu, C., Muller Hermelink, H.K. and Wallich, R. (1991) Recombinant outer surface protein A from *Borrelia burgdorferi* induces antibodies protective against spirochaetal infection in mice. J. Infect. Dis. 164, 123–132.
[11] Fikrig, E., Barthold, S.W., Marcantonio, N., Deponte, K., Kantor, F.S. and Flavell, R.A. (1992) Roles of OspA, OspB, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. Infect. Immun. 60, 657–661.
[12] Fikrig, E., Telford, S.R., Barthold, S.W., Kantor, F.S., Spielman, A. and Flavell, R.A. (1992) Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. Proc. Natl. Acad. Sci. USA 89, 5418–5421.
[13] Gern, L., Rais, O., Capiau, C., Hauser, P., Lobet, Y., Simoen, E., Voet, P. and Petre, J. (1994) Immunization of mice by recombinant OspA preparations and protection against *Borrelia burgdorferi* infection induced by *Ixodes ricinus* tick bites. Immunol. Lett. 39, 249–258.
[14] Lim, L.C., England, D.M., DuChateau, B.K., Glowacki, N.J., Creson, J.R., Lovrich, S.D., Callister, S.M., Jobe, D.A. and Schell, R.F. (1994) Development of destructive arthritis in vaccinated hamsters challenged with *Borrelia burgdorferi*. Infect. Immun. 62, 2825–2833.
[15] Steere, A.C., Dwyer, E. and Winchester, R. (1990) Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. New Engl. J. Med. 323, 219–223.
[16] Lengl Janssen, B., Strauss, A.F., Steere, A.C. and Kamradt, T. (1994) The T helper cell response in Lyme arthritis: differential recognition of *Borrelia burgdorferi* outer surface protein A in patients with treatment-resistant or treatment-responsive Lyme arthritis. J. Exp. Med. 180, 2069–2078.
[17] Kamradt, T., Lengl Janssen, B., Strauss, A.F., Bansal, G. and Steere, A.C. (1996) Dominant recognition of a *Borrelia burgdorferi* outer surface protein A peptide by T helper cells in patients with treatment-resistant Lyme arthritis. Infect. Immun. 64, 1284–1289.
[18] Kamradt, T., Krause, A. and Burmester, G.R. (1995) A role for T cells in the pathogenesis of treatment-resistant Lyme arthritis. Mol. Med. 1, 486–490.
[19] Sears, J.E., Fikrig, E., Nakagawa, T.Y., Deponte, K., Marcantonio, N., Kantor, F.S. and Flavell, R.A. (1991) Molecular mapping of Osp-A mediated immunity against *Borrelia burgdorferi*, the agent of Lyme disease. J. Immunol. 147, 1995–2000.
[20] Shanafelt, M.C., Anzola, J., Soderberg, C., Yssel, H., Turck, C.W. and Peltz, G. (1992) Epitopes on the outer surface protein A of *Borrelia burgdorferi* recognized by antibodies and T cells of patients with Lyme disease. J. Immunol. 148, 218–224.
[21] Krause, A., Burmester, G.R., Rensing, A., Schoerner, C., Schaible, U.E., Simon, M.M., Herzer, P., Kramer, M.D. and Wallich, R. (1992) Cellular immune reactivity to recombinant OspA and flagellin from *Borrelia burgdorferi* in patients with Lyme borreliosis. Complexity of humoral and cellular immune responses. J. Clin. Invest. 90, 1077–1084.
[22] Dillon, S.B., Demuth, S.G., Schneider, M.A., Weston, C.B., Jones, C.S., Young, J.F., Scott, M., Bhatnaghar, P.K., LoCastro, S. and Hanna, N. (1992) Induction of protective class I MHC-restricted CTL in mice by a recombinant influenza vaccine in aluminium hydroxide adjuvant. Vaccine 10, 309–318.
[23] Gordon, D.M. (1993) Use of novel adjuvants and delivery systems to improve the humoral and cellular immune response to malaria vaccine candidate antigens. Vaccine 11, 591–593.
[24] Yamada, A., Ziese, M.R., Young, J.F., Yamada, Y.K. and Ennis, F.A. (1985) Influenza virus hemagglutinin-specific cy-

totoxic T cell response induced by polypeptide produced in *Escherichia coli*. J. Exp. Med. 162, 663–674.

[25] Fries, L.F., Dillon, S.B., Hildreth, J.E., Karron, R.A., Funkhouser, A.W., Friedman, C.J., Jones, C.S., Culleton, V.G. and Clements, M.L. (1993) Safety and immunogenicity of a recombinant protein influenza A vaccine in adult human volunteers and protective efficacy against wild-type H1N1 virus challenge. J. Infect. Dis. 167, 593–601.

[26] Erdile, L.F., Brandt, M.A., Warakomski, D.J., Westrack, G.J., Sadziene, A., Barbour, A.G. and Mays, J.P. (1993) Role of attached lipid in immunogenicity of *Borrelia burgdorferi* OspA. Infect. Immun. 61, 81–90.

[27] Schild, H., Deres, K., Wiesmuller, K.H., Jung, G. and Rammensee, H.G. (1991) Efficiency of peptides and lipopeptides for in vivo priming of virus-specific cytotoxic T cells. Eur. J. Immunol. 21, 2649–2654.

[28] Bessler, W.G., Suhr, B., Buhring, H.J., Muller, C.P., Wiesmuller, K.H., Becker, G. and Jung, G. (1985) Specific antibodies elicited by antigen covalently linked to a synthetic adjuvant. Immunobiology 170, 239–244.

[29] Gupta, R.K., Relyveld, E.H., Lindblad, E.B., Bizzini, B., Ben-Efraim, S. and Gupta, C.K. (1993) Adjuvants – a balance between toxicity and adjuvanticity. Vaccine 11, 293–306.

[30] Hurtenbach, U., Museteanu, C., Gasser, J., Schaible, U.E. and Simon, M.M. (1995) Studies on early events of *Borrelia burgdorferi*-induced cytokine production in immunodeficient SCID mice by using a tissue chamber model for acute inflammation. Int. J. Exp. Pathol. 76, 111–123.

[31] Tai, K.F., Ma, Y. and Weis, J.J. (1994) Normal human B lymphocytes and mononuclear cells respond to the mitogenic and cytokine-stimulatory activities of *Borrelia burgdorferi* and its lipoprotein OspA. Infect. Immun. 62, 520–528.

[32] Ma, Y. and Weis, J.J. (1993) *Borrelia burgdorferi* outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine-stimulatory properties. Infect. Immun. 61, 3843–3853.

[33] Radolf, J.D., Arndt, L.L., Akins, D.R., Curetty, L.L., Levi, M.E., Shen, Y., Davis, L.S. and Norgard, M.V. (1995) *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides activate monocytes/macrophages. J. Immunol. 154, 2866–2877.

[34] Braun, V. (1975) Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta 415, 335–377.

[35] Johnson, R.B., Kohl, S. and Bessler, W.G. (1983) Polyclonal activation of B-lymphocytes in vivo by *Salmonella typhimurium* lipoprotein. Infect. Immun. 39, 1481–1484.

[36] Bessler, W.G., Cox, M., Lex, A., Suhr, B., Wiesmuller, K.H. and Jung, G. (1985) Synthetic lipopeptide analogs of bacterial lipoprotein are potent polyclonal activators for murine B lymphocytes. J. Immunol. 135, 1900–1905.

[37] Hoffmann, P., Heinle, S., Schade, U.F., Loppnow, H., Ulmer, A.J., Flad, H.D., Jung, G. and Bessler, W.G. (1988) Stimulation of human and murine adherent cells by bacterial lipoprotein and synthetic lipopeptide analogues. Immunobiology 177, 158–170.

[38] Johnson, R.B., Kohl, S., Wiesmuller, K., Jung, G. and Bessler, W.G. (1983) Synthetic analogues of the N-terminal lipid part of bacterial lipoprotein are B-lymphocyte mitogens in vitro and in vivo. Immunobiology 165, 27–35.

[39] Deres, K., Schild, H., Wiesmuller, K.H., Jung, G. and Rammensee, H.G. (1989) In vivo priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. Nature 342, 561–564.

[40] Wolf, B., Uhl, B., Hauschildt, S., Metzger, J., Jung, G. and Bessler, W.G. (1989) Interaction of the lymphoid cell line BCL1 with lipopeptide analogues of bacterial lipoprotein: electron energy loss spectroscopy (EELS) as a novel method to detect the distribution of the activator within the cells. Immunobiology 180, 93–100.

[41] Wolf, B., Hauschildt, S., Uhl, B., Metzger, J., Jung, G. and Bessler, W.G. (1989) Localization of the cell activator lipopeptide in bone marrow-derived macrophages by electron energy loss spectroscopy (EELS). Immunol. Lett. 20, 121–126.

[42] Wiesmuller, K.H., Jung, G. and Hess, G. (1989) Novel low-molecular-weight synthetic vaccine against foot-and-mouth disease containing a potent B-cell and macrophage activator. Vaccine 7, 29–33.

[43] Chedid, L. (1985) Adjuvants of immunity. Ann. Inst. Pasteur Immunol. 136D, 283–291.

[44] Tagliabue, A. and Boraschi, D. (1993) Cytokines as vaccine adjuvants: interleukin 1 and its synthetic peptide 163–171. Vaccine 11, 594–595.

[45] Van Hoecke, C., De Grave, D., Hauser, P. and Lebacq, E. (1994) Evaluation of three formulations of a candidate vaccine against Lyme disease in healthy adult volunteers. In: Advances in Lyme Borreliosis: Proceedings of the VI International Conference on Lyme Borreliosis (Cevenini, R., Sambri, V. and La Placa, M., Eds.), pp. 123–126. Esculapio, Bologna.

[46] Nocton, J.J., Dressler, F., Rutledge, B.J., Rys, P.N., Persing, D.H. and Steere, A.C. (1994) Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. New Engl. J. Med. 330, 229–234.

[47] Fikrig, E., Barthold, S.W. and Flavell, R.A. (1993) OspA vaccination of mice with established *Borrelia burgdorferi* infection alters disease but not infection. Infect. Immun. 61, 2553–2557.

[48] Schoen, R.T., Meurice, F., Brunet, C.M., Cretella, S., Krause, D.S., Craft, J.E. and Fikrig, E. (1995) Safety and immunogenicity of an outer surface protein A vaccine in subjects with previous Lyme disease. J. Infect. Dis. 172, 1324–1329.